Patent
600-1-195C

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS:	JAMES E. DARNELL, JR. ET AL.		
SERIAL NO. :	UNASSIGNED	EXAMINER :	UNKNOWN
FILED :	HEREWITH	ART UNIT :	UNKNOWN
FOR :	NUCLEIC ACIDS ENCODING RECEPTOR RECOGNITION FACTORS, AND METHODS OF USE THEREOF (AMENDED)		

VIA EXPRESS MAIL: EL 920251268 US
DATE OF DEPOSIT: JUNE 7, 2001

PRELIMINARY AMENDMENT

ASSISTANT COMMISSIONER FOR PATENTS
Box PATENT APPLICATION
WASHINGTON, D.C. 20231

Dear Sir:

In accordance with Rule 111 of the Rules of Practice please consider the following amendments and remarks.

IN THE SPECIFICATION:

Please replace the paragraph beginning on Page 1, line 6 directly under the subtitle: "CROSS-REFERENCE TO RELATED APPLICATIONS " with:

- - The present Application is a Continuation of copending U.S. Ser. No. 09/488,442 filed January 20, 2000 which is a Continuation of copending U.S. Ser. No. 08/948,547, filed October 10, 1997, and issued as U.S. Pat. No. 6,124,118, which is a Continuation of U.S. Serial No. 08/820,754, filed Mar. 19, 1997 and issued as U.S. Pat. No. 5,976,835, which is a Division of U.S. Ser. No. 08/212,185, filed Mar. 11, 1994 which is a Continuation-In-Part of U.S. Ser. No. 08/126,588 and U.S. Ser. No. 08/126,595, both filed Sep. 24, 1993, both now abandoned, which are both Continuations-In-Part of U.S. Ser. No. 07/980,498, filed Nov. 23, 1992, now abandoned, which is a Continuation-In-Part of U.S. Ser. No. 07/854,296, filed Mar. 19, 1992, now abandoned, the disclosures of which are hereby incorporated by reference

in their entireties. Applicants claim the benefits of these Applications under 35 U.S.C. §120.-
-

Please replace the last paragraph on Page 5 with:

- - The recognition factor is now known to comprise several proteinaceous substituents, in the instance of IFNα and IFNγ. Particularly, three proteins derived from the factor ISGF-3 have been successfully sequenced and their sequences are set forth in FIGURE 1 (SEQ ID NOS:1, 2), FIGURE 2 (SEQ ID NOS:3, 4) and FIGURE 3 (SEQ. ID NOS.5, 6) herein. Additionally, a murine gene encoding the 91 kD protein (*i.e.*, the murine homologue of the human protein having an amino acid sequence of SEQ ID NO:4) has been identified and sequenced. The nucleotide sequence (SEQ ID NO:7) and deduced amino acid sequence (SEQ ID NO:8) are shown in FIGURE 13A-13C. - -

Please replace the second full paragraph of Page 7, beginning on line 9 with:

- - In a specific example, the receptor recognition factor represented by SEQ ID NO:4 possesses the added capability of acting as a transcription factor and, in particular, as a DNA binding protein in response to interferon-γ stimulation. This discovery presages an expanded role for the proteins in question, and other proteins and like factors that have heretofore been characterized as receptor recognition factors. It is therefore apparent that a single factor may indeed provide the nexus between the liganded receptor at the cell surface and direct participation in DNA transcriptional activity in the nucleus. This pleiotypic factor has the following characteristics:

a) It interacts with an interferon-γ-bound receptor kinase complex;

b) It is a tyrosine kinase substrate; and

c) When phosphorylated, it serves as a DNA binding protein. - -

Please replace the bridging paragraph between Pages 8 and 9, beginning on line 29 of Page 8 with:

- - The present invention also relates to a recombinant DNA molecule or cloned gene, or a degenerate variant thereof, which encodes a receptor recognition factor, or a fragment

thereof, that possesses a molecular weight of about 113 kD and an amino acid sequence set forth in FIGURE 1 (SEQ ID NO:2); preferably a nucleic acid molecule, in particular a recombinant DNA molecule or cloned gene, encoding the 113 kD receptor recognition factor has a nucleotide sequence or is complementary to a DNA sequence shown in FIGURE 1 (SEQ ID NO:1). In another embodiment, the receptor recognition factor has a molecular weight of about 91 kD and the amino acid sequence set forth in FIGURE 2 (SEQ ID NO:4) or FIGURE 13 (SEQ ID NO:8); preferably a nucleic acid molecule, in particular a recombinant DNA molecule or cloned gene, encoding the 91 kD receptor recognition factor has a nucleotide sequence or is complementary to a DNA sequence shown in FIGURE 2 (SEQ ID NO:3) or FIGURE 13 (SEQ ID NO:8). In yet a further embodiment, the receptor recognition factor has a molecular weight of about 84 kD and the amino acid sequence set forth in FIGURE 3 (SEQ ID NO:6); preferably a nucleic acid molecule, in particular a recombinant DNA molecule or cloned gene, encoding the 84 kD receptor recognition factor has a nucleotide sequence or is complementary to a DNA sequence shown in FIGURE 3 (SEQ ID NO:5). In yet another embodiment, the receptor recognition factor has an amino acid sequence set forth in FIGURE 14 (SEQ ID NO:10); preferably a nucleic acid molecule, in particular a recombinant DNA molecule or cloned gene, encoding such receptor recognition factor has a nucleotide sequence or is complementary to a DNA sequence shown in FIGURE 14 (SEQ ID NO:9). In still another embodiment, the receptor recognition factor has an amino acid sequence set forth in FIGURE 15 (SEQ ID NO:12); preferably a nucleic acid molecule, in particular a recombinant DNA molecule or cloned gene, encoding such receptor recognition factor has a nucleotide sequence or is complementary to a DNA sequence shown in FIGURE 15 (SEQ ID NO:11). - -

Please replace the fifth paragraph of Page 17, beginning on line 23 with:

- - FIGURE 1A-1E depicts the full receptor recognition factor nucleic acid sequence and the deduced amino acid sequence derived for the ISGF-3α gene defining the 113 kD protein. The nucleotides are numbered from 1 to 2553 (SEQ ID NO:1), and the amino acids are numbered from 1 to 851 (SEQ ID NO:2). - -

Please replace the sixth paragraph of Page 17, beginning on line 28 with:

- - FIGURE 2A-2D depicts the full receptor recognition factor nucleic acid sequence and the deduced amino acid sequence derived for the ISGF-3α gene defining the 91 kD protein. The nucleotides are numbered from 1 to 3943 (SEQ ID NO:3), and the amino acids are numbered from 1 to 750 (SEQ ID NO:4). - -

Please replace the first paragraph of Page 18, beginning on line 1 with:

- - FIGURE 3A-3C depicts the full receptor recognition factor nucleic acid sequence and the deduced amino acid sequence derived for the ISGF-3α gene defining the 84 kD protein. The nucleotides are numbered from 1 to 2166 (SEQ ID NO:5), and the amino acids are numbered from 1 to 712 (SEQ ID NO:6). - -

Please replace the third paragraph of Page 18, beginning on line 19 with:

- - FIGURE 5a-5b generally presents the results of Northern Blot analysis for the 91/84 kD peptides. Figure 5a presents restriction maps for cDNA clones E4 (top map) and E3 (bottom map) showing DNA fragments that were radiolabeled as probes (probes A-D). Figure 5b comprises Northern blots of cytoplasmic HeLa RNA hybridized with the indicated probes. The 4.4 and 3.1 KB species as well as the 28S and 18S rRNA bands are indicated. - -

Please replace the first full paragraph of Page 19, beginning on line 4 with:

- - FIGURE 7a-7e presents the results of Western blot and antibody shift analyses.

a) Highly purified ISGF-3, fractionated on a 7.0% SDS polyacrylamide gel, was probed with antibodies a42 (amino acids 597-703); a55 (amino acids 2-59); and a57 (amino acids 705-739) in a Western blot analysis. The silver stained part of the gel (lanes a, b, and c) illustrates the location of the ISGF-3 component proteins and the purity of the material used in Western blot: Lane a) Silver stain of protein sample used in all the Western blot experiments (immune and preimmune). Lane b) Material of equal purity to that shown in Fig. 4, for clearer identification of the ISGF-3 proteins. Lane c) Size protein markers indicated.

b) Antibody interference of the ISGF-3 shift complex; Lane a) The complete ISGF-3 and the free ISGF-3γ component shift with partially purified ISGF-3 are marked; Lane b) Competition with a 100 fold excess of cold ISRE oligonucleotide. Lane c) Shift complex after the addition of 1 ml of preimmune serum to a 12.5 µl shift reaction. Lanes d and e) - Shift complex after the addition of 1 µl of a 1:10 dilution or 1 ml of undiluted a42 antiserum to a 12.5 µl shift reaction. - -

Please replace the first full paragraph of Page 23, beginning on line 4 with:

- -FIGURE 13 depicts (A) the deduced amino acid sequence (SEQ ID NO:8) of and (B- C) the DNA sequence (SEQ ID NO:7) encoding the murine 91 kD intracellular receptor recognition factor. - -

Please replace the second full paragraph of Page 23, beginning on line 8 with:

- - FIGURE 14 depicts (A) the deduced amino acid sequence (SEQ ID NO:10) of and (B-D) the DNA sequence (SEQ ID NO:9) encoding the 13sf1 intracellular receptor recognition factor. - -

Please replace the third full paragraph of Page 23, beginning on line 12 with:

- - FIGURE 15 depicts (A) the deduced amino acid sequence (SEQ ID NO:12) of and (B-E) the DNA sequence (SEQ ID NO:11) encoding the 19sf6 intracellular receptor recognition factor. - -

Please replace the bridging paragraph between Pages 37 and 38, beginning on line 23 of Page 37 with:

- - As stated above, the present invention also relates to a recombinant DNA molecule or cloned gene, or a degenerate variant thereof, which encodes a receptor recognition factor, or a fragment thereof, that possesses a molecular weight of about 113 kD and an amino acid sequence set forth in FIGURE 1 (SEQ ID NO:2); preferably a nucleic acid molecule, in particular a recombinant DNA molecule or cloned gene, encoding the 113 kD receptor recognition factor has a nucleotide sequence or is complementary to a DNA sequence shown

in FIGURE 1 (SEQ ID NO:1). In another embodiment, the receptor recognition factor has a molecular weight of about 91 kD and the amino acid sequence set forth in FIGURE 2 (SEQ ID NO:4) or FIGURE 13 (SEQ ID NO:8); preferably a nucleic acid molecule, in particular a recombinant DNA molecule or cloned gene, encoding the 91 kD receptor recognition factor has a nucleotide sequence or is complementary to a DNA sequence shown in FIGURE 2 (SEQ ID NO:3) or FIGURE 13 (SEQ ID NO:8). In yet a further embodiment, the receptor recognition factor has a molecular weight of about 84 kD and the amino acid sequence set forth in FIGURE 3 (SEQ ID NO:6); preferably a nucleic acid molecule, in particular a recombinant DNA molecule or cloned gene, encoding the 84 kD receptor recognition factor has a nucleotide sequence or is complementary to a DNA sequence shown in FIGURE 3 (SEQ ID NO:5). In yet another embodiment, the receptor recognition factor has an amino acid sequence set forth in FIGURE 14 (SEQ ID NO:10); preferably a nucleic acid molecule, in particular a recombinant DNA molecule or cloned gene, encoding such receptor recognition factor has a nucleotide sequence or is complementary to a DNA sequence shown in FIGURE 14 (SEQ ID NO:9). In still another embodiment, the receptor recognition factor has an amino acid sequence set forth in FIGURE 15 (SEQ ID NO:12); preferably a nucleic acid molecule, in particular a recombinant DNA molecule or cloned gene, encoding such receptor recognition factor has a nucleotide sequence or is complementary to a DNA sequence shown in FIGURE 15 (SEQ ID NO:11). - -

Please replace the bridging paragraph between Pages 69 and 70, beginning on line 30 of Page 69 with:

- - A fragment of the gene encoding the human 91 kD protein was used to screen a murine thymus and spleen cDNA library for homologous proteins. The screening assay yielded a highly homologous gene encoding a murine polypeptide that is greater than 95% homologous to the human 91 kD protein. The nucleic acid and deduced amino acid sequence of the murine 91 kD protein are shown in Figure13A-13C, and SEQ ID NO:7 (nucleotide sequence) and SEQ ID NO:8 (amino acid sequence). - -

Please replace the subtitle on Page 76, line 11, following the second paragraph with:

- - EXAMPLE 6: DIMERIZATION OF PHOSPHORYLATED STAT91 - -

Please amend the title of the application to read:

- - NUCLEIC ACIDS ENCODING RECEPTOR RECOGNITION FACTORS AND METHODS OF USE THEREOF - -

Applicants request that the Specification be amended to include the Sequence Listing submitted herewith.

IN THE CLAIMS:

Please cancel Claims 2-68 without prejudice:

Please add the following new claims.

--69. A recombinant DNA molecule encoding a receptor recognition factor (RRF) protein having the following characteristics:

a) said RRF is cytoplasmic in origin;

b) said RRF is activated by tyrosine phosphorylation;

c) upon activation said RRF is translocated to the nucleus of a target cell; and

d) said RRF has an amino acid sequence comprising a sequence of contiguous amino acid residues which is present in both SEQ ID NO:2 and SEQ ID NO:4; wherein the sequence of contiguous amino acid residues contains four or more consecutive amino acids.

70. The recombinant DNA molecule of Claim 69 wherein the sequence of continguous amino acid residues contains four or more consecutive amino acids and is selected from the group consisting of:

a) HQLY (amino acids 19-22 of SEQ ID NO:2 and 19-22 of SEQ ID NO4);

b) IRQY (amino acids 31-34 of SEQ ID NO:2 and 30-33 of SEQ ID NO4);

c) RQYL (amino acids 32-35 of SEQ ID NO:2 and 31-34 of SEQ ID NO4);

d) LLQH (amino acids 82-85 of SEQ ID NO:2 and 78-81 of SEQ ID NO4);

e) LQHN (amino acids 83-86 of SEQ ID NO:2 and 79-82 of SEQ ID NO4);

f) RKEV (amino acids 210-213 of SEQ ID NO:2 and 210-213 of SEQ ID NO4);

g) FVVE (amino acids 316-319 of SEQ ID NO:2 and 317-320 of SEQ ID NO4);

h) QPCM (amino acids 321-324 of SEQ ID NO:2 and 322-325 of SEQ ID NO4);

i) PCMP (amino acids 322-325 of SEQ ID NO:2 and 323-326 of SEQ ID NO4);

j) LKTG (amino acids 334-337 of SEQ ID NO:2 and 335-338 of SEQ ID NO:4);

k) RLLV (amino acids 345-348 of SEQ ID NO:2 and 346-349 of SEQ ID NO:4);

l) GFRK (amino acids 372-375 of SEQ ID NO:2 and 376-379 of SEQ ID NO:4);

m) FRKF (amino acids 373-376 of SEQ ID NO:2 and 377-380 of SEQ ID NO:4);

n) RKFN (amino acids 374-377 of SEQ ID NO:2 and 378-381 of SEQ ID NO:4);

o) KFNI (amino acids 375-378 of SEQ ID NO:2 and 379-382 of SEQ ID NO:4);

p) FNIL (amino acids 376-379 of SEQ ID NO:2 and 380-383 of SEQ ID NO:4);

q) VTEE (amino acids 424-427 of SEQ ID NO:2 and 426-429 of SEQ ID NO:4);

r) TEEL (amino acids 425-428 of SEQ ID NO:2 and 427-430 of SEQ ID NO:4);

s) EELH (amino acids 426-429 of SEQ ID NO:2 and 428-431 of SEQ ID NO:4);

t) LPVV (amino acids 451-454 of SEQ ID NO:2 and 453-456 of SEQ ID NO:4);

u) LSWQ (amino acids 500-503 of SEQ ID NO:2 and 502-505 of SEQ ID NO:4);

v) SWQF (amino acids 501-504 of SEQ ID NO:2 and 503-506 of SEQ ID NO:4);

w) WQFS (amino acids 502-505 of SEQ ID NO:2 and 504-507 of SEQ ID NO:4);

x) QFSS (amino acids 503-506 of SEQ ID NO:2 and 505-508 of SEQ ID NO:4);

y) RGLN (amino acids 510-513 of SEQ ID NO:2 and 512-515 of SEQ ID NO:4);

z) ILEL (amino acids 560-563 of SEQ ID NO:2 and 561-564 of SEQ ID NO:4);

zz) LWND (amino acids 571-574 of SEQ ID NO:2 and 572-575 of SEQ ID NO:4);

aa) WNDG (amino acids 572-575 of SEQ ID NO:2 and 573-576 of SEQ ID NO:4);

bb) IMGF (amino acids 577-580 of SEQ ID NO:2 and 578-581 of SEQ ID NO:4);

cc) GTFL (amino acids 596-599 of SEQ ID NO:2 and 597-600 of SEQ ID NO:4);

dd) TFLL (amino acids 597-600 of SEQ ID NO:2 and 598-601 of SEQ ID NO:4);

ee) FLLR (amino acids 598-601 of SEQ ID NO:2 and 599-602 of SEQ ID NO:4);

ff) LLRF (amino acids 599-602 of SEQ ID NO:2 and 600-603 of SEQ ID NO:4);

gg) LRFS (amino acids 600-603 of SEQ ID NO:2 and 601-604 of SEQ ID NO:4);

hh) RFSE (amino acids 601-604 of SEQ ID NO:2 and 602-605 of SEQ ID NO:4);

ii) FSES (amino acids 602-605 of SEQ ID NO:2 and 603-606 of SEQ ID NO:4);

jj) SESS (amino acids 603-606 of SEQ ID NO:2 and 604-607 of SEQ ID NO:4);

kk) PYTK (amino acids 630-633 of SEQ ID NO:2 and 633-636 of SEQ ID NO:4);

ll) ENIP (amino acids 654-657 of SEQ ID NO:2 and 657-660 of SEQ ID NO:4);

mm) NIPE (amino acids 655-658 of SEQ ID NO:2 and 658-661 of SEQ ID NO:4);

nn) IPEN (amino acids 656-659 of SEQ ID NO:2 and 659-662 of SEQ ID NO:4);

oo) PENP (amino acids 657-660 of SEQ ID NO:2 and 660-663 of SEQ ID NO:4); and

pp) ENPL (amino acids 658-661 of SEQ ID NO:2 and 661-664 of SEQ ID NO:4).

71. The recombinant DNA molecule of Claim 70 wherein the sequence of contiguous amino acid residues contains five or more consecutive amino acids and is selected from the group consisting of:

a) IRQYL (amino acids 31-35 of SEQ ID NO:2 and 30-34 of SEQ ID NO:4);

b) LLQHN (amino acids 82-86 of SEQ ID NO:2 and 78-82 of SEQ ID NO:4);

c) QPCMP (amino acids 321-325 of SEQ ID NO:2 and 322-326 of SEQ ID NO:4);

d) GFRKF (amino acids 372-376 of SEQ ID NO:2 and 376-380 of SEQ ID NO:4);

e) FRKFN (amino acids 373-377 of SEQ ID NO:2 and 377-381 of SEQ ID NO:4);

f) RKFNI (amino acids 374-378 of SEQ ID NO:2 and 378-382 of SEQ ID NO:4);

g) KFNIL (amino acids 375-379 of SEQ ID NO:2 and 379-383 of SEQ ID NO:4);

h) VTEEL (amino acids 424-428 of SEQ ID NO:2 and 426-430 of SEQ ID NO:4);

i) TEELH (amino acids 425-429 of SEQ ID NO:2 and 427-431 of SEQ ID NO:4);

j) LSWQF (amino acids 500-504 of SEQ ID NO:2 and 502-506 of SEQ ID NO:4);

k) SWQFS (amino acids 501-505 of SEQ ID NO:2 and 503-507 of SEQ ID NO:4);

l) WQFSS (amino acids 502-506 of SEQ ID NO:2 and 504-508 of SEQ ID NO:4);

m) LWNDG (amino acids 571-575 of SEQ ID NO:2 and 572-576 of SEQ ID NO:4);

n) GTFLL (amino acids 596-600 of SEQ ID NO:2 and 597-601 of SEQ ID NO:4);

o) TFLLR (amino acids 597-601 of SEQ ID NO:2 and 598-602 of SEQ ID NO:4);

p) FLLRF (amino acids 598-602 of SEQ ID NO:2 and 599-603 of SEQ ID NO:4);

q) LLRFS (amino acids 599-603 of SEQ ID NO:2 and 600-604 of SEQ ID NO:4);

r) LRFSE (amino acids 600-604 of SEQ ID NO:2 and 601-605 of SEQ ID NO:4);

s) RFSES (amino acids 601-605 of SEQ ID NO:2 and 602-606 of SEQ ID NO:4);

t) FSESS (amino acids 602-606 of SEQ ID NO:2 and 603-607 of SEQ ID NO:4);

u) ENIPE (amino acids 654-658 of SEQ ID NO:2 and 657-661 of SEQ ID NO:4);

v) NIPEN (amino acids 655-659 of SEQ ID NO:2 and 658-662 of SEQ ID NO:4);

w) IPENP (amino acids 656-660 of SEQ ID NO:2 and 659-663 of SEQ ID NO:4); and

x) PENPL (amino acids 657-661 of SEQ ID NO:2 and 660-664 of SEQ ID NO:4).

72. The recombinant DNA molecule of Claim 71 wherein the sequence of contiguous amino acid residues contains six or more consecutive amino acids and is selected from the group consisting of:

a) GFRKFN (amino acids 372-377 of SEQ ID NO:2 and 376-381 of SEQ ID NO:4);

b) FRKFNI (amino acids 373-378 of SEQ ID NO:2 and 377-382 of SEQ ID NO:4);

c) RKFNIL (amino acids 374-379 of SEQ ID NO:2 and 378-383 of SEQ ID NO:4);

d) VTEELH (amino acids 424-429 of SEQ ID NO:2 and 426-431 of SEQ ID NO:4);

e) LSWQFS (amino acids 500-505 of SEQ ID NO:2 and 502-507 of SEQ ID NO:4);

f) SWQFSS (amino acids 501-506 of SEQ ID NO:2 and 503-508 of SEQ ID NO:4);

g) GTFLLR (amino acids 596-601 of SEQ ID NO:2 and 597-602 of SEQ ID NO:4);

h) TFLLRF (amino acids 597-602 of SEQ ID NO:2 and 598-603 of SEQ ID NO:4);

i) FLLRFS (amino acids 598-603 of SEQ ID NO:2 and 599-604 of SEQ ID NO:4);

j) LLRFSE (amino acids 599-604 of SEQ ID NO:2 and 600-605 of SEQ ID NO:4);

k) LRFSES (amino acids 600-605 of SEQ ID NO:2 and 601-606 of SEQ ID NO:4);

l) RFSESS (amino acids 601-606 of SEQ ID NO:2 and 602-607 of SEQ ID NO:4);

m) ENIPEN (amino acids 654-659 of SEQ ID NO:2 and 657-662 of SEQ ID NO:4);

n) NIPENP (amino acids 655-660 of SEQ ID NO:2 and 658-663 of SEQ ID NO:4); and

o) IPENPL (amino acids 656-661 of SEQ ID NO:2 and 659-664 of SEQ ID NO:4).

73. The recombinant DNA molecule of Claim 72 wherein the sequence of contiguous amino acid residues contains seven or more consecutive amino acids and is selected from the group consisting of:

a) GFRKFNI (amino acids 372-378 of SEQ ID NO:2 and 376-382 of SEQ ID NO:4);

b) FRKFNIL (amino acids 373-379 of SEQ ID NO:2 and 377-383 of SEQ ID NO:4);

c) LSWQFSS (amino acids 500-506 of SEQ ID NO:2 and 502-508 of SEQ ID NO:4);

d) GTFLLRF (amino acids 596-602 of SEQ ID NO:2 and 597-603 of SEQ ID NO:4);

e) TFLLRFS (amino acids 597-603 of SEQ ID NO:2 and 598-604 of SEQ ID NO:4);

f) FLLRFSE (amino acids 598-604 of SEQ ID NO:2 and 599-605 of SEQ ID NO:4);

g) LLRFSES (amino acids 599-605 of SEQ ID NO:2 and 600-606 of SEQ ID NO:4);

h) LRFSESS (amino acids 600-606 of SEQ ID NO:2 and 601-607 of SEQ ID NO:4);

i) ENIPENP (amino acids 654-660 of SEQ ID NO:2 and 657-663 of SEQ ID NO:4); and

j) NIPENPL (amino acids 655-661 of SEQ ID NO:2 and 658-664 of SEQ ID NO:4).

74. The recombinant DNA molecule of Claim 73 wherein the sequence of contiguous amino acid residues contains eight or more consecutive amino acids and is selected from the group consisting of:

a) GFRKFNIL (amino acids 372-379 of SEQ ID NO:2 and 376-383 of SEQ ID NO:4);

b) GTFLLRFS (amino acids 596-603 of SEQ ID NO:2 and 597-604 of SEQ ID NO:4);

c) TFLLRFSE (amino acids 597-604 of SEQ ID NO:2 and 598-605 of SEQ ID NO:4);

d) FLLRFSES (amino acids 598-605 of SEQ ID NO:2 and 599-606 of SEQ ID NO:4);

e) LLRFSESS (amino acids 599-606 of SEQ ID NO:2 and 600-607 of SEQ ID NO:4); and

f) ENIPENPL (amino acids 654-661 of SEQ ID NO:2 and 657-664 of SEQ ID NO:4).

75. The recombinant DNA molecule of Claim 74 wherein the sequence of contiguous amino acid residues contains nine or more consecutive amino acids and is selected from the group consisting of:

a) GTFLLRFSE (amino acids 596-604 of SEQ ID NO:2 and 597-605 of SEQ ID NO:4);

b) TFLLRFSES (amino acids 597-605 of SEQ ID NO:2 and 598-606 of SEQ ID NO:4); and

c) FLLRFSESS (amino acids 598-606 of SEQ ID NO:2 and 599-607 of SEQ ID NO:4).

76. The recombinant DNA molecule of Claim 75 wherein the sequence of contiguous amino acid residues contains ten or more consecutive amino acids and is selected from the group consisting of:

a) GTFLLRFSES (amino acids 596-605 of SEQ ID NO:2 and 597-606 of SEQ ID NO:4); and

b) TFLLRFSESS (amino acids 597-606 of SEQ ID NO:2 and 598-607 of SEQ ID NO:4).

77. The recombinant DNA molecule of Claim 76 wherein the sequence of continguous amino acid residues contains eleven consecutive amino acids having the sequence GTFLLRFSESS (amino acids 596-606 of SEQ ID NO:2 and 597-607 of SEQ ID NO:4).

78. The recombinant DNA molecule of Claim 70 wherein said RRF has an amino acid sequence which further comprises a second sequence of contiguous amino acid residues, wherein the second sequence of contiguous amino acid residues also contains four or more consecutive amino acids which is present in both SEQ ID NO:2 and SEQ ID NO:4.

79. A recombinant DNA molecule encoding a receptor recognition factor (RRF) protein having the following characteristics:

a) said RRF is cytoplasmic in origin;

b) said RRF is activated by tyrosine phosphorylation; and

c) upon activation said RRF is translocated to the nucleus of a target cell, wherein said DNA molecule hybridizes to the nucleotide sequence set forth in SEQ ID NO:1 under standard hybridization conditions.

80. A recombinant DNA molecule encoding a receptor recognition factor (RRF) protein having the following characteristics:

a) said RRF is cytoplasmic in origin;

b) said RRF is activated by tyrosine phosphorylation; and

c) upon activation said RRF is translocated to the nucleus of a target cell; wherein said DNA molecule hybridizes to the nucleotide sequence set forth in SEQ ID NO:3 under standard hybridization conditions.

81. A recombinant DNA molecule encoding a receptor recognition factor (RRF) protein having the following characteristics:

(a) the RRF is cytoplasmic in origin;

(b) the RRF is activated by tyrosine phosphorylation; and

(c) upon activation said RRF is translocated to the nucleus of a target cell;

wherein the RRF contains one or more of the boxed regions in Figure 8B.

82 The recombinant DNA molecule of Claim 81, wherein the RRF further contains a tyrosyl residue at a position that corresponds to the conserved position identified in SEQ ID NO:2 and SEQ ID NO:4, said position selected from the group consisting of:

amino acid 22 of SEQ ID NO:2 and amino acid 22 of SEQ ID NO:4;
amino acid 34 of SEQ ID NO:2 and amino acid 33 of SEQ ID NO:4;
amino acid 288 of SEQ ID NO:2 and amino acid 289 of SEQ ID NO:4;
amino acid 631 of SEQ ID NO:2 and amino acid 634 of SEQ ID NO:4;
amino acid 648 of SEQ ID NO:2 and amino acid 651 of SEQ ID NO:4;
amino acid 665 of SEQ ID NO:2 and amino acid 668 of SEQ ID NO:4;
amino acid 677 of SEQ ID NO:2 and amino acid 680 of SEQ ID NO:4;
amino acid 678 of SEQ ID NO:2 and amino acid 681 of SEQ ID NO:4; and
amino acid 690 of SEQ ID NO:2 and amino acid 701 of SEQ ID NO:4.

83. The recombinant DNA molecule of Claim 81 wherein the RRF comprises a highly negative charged domain at its C-terminal end.

84. The recombinant DNA molecule of Claim 81 wherein the RRF comprises an SH2 domain.

85. The recombinant DNA molecule of Claim 84 wherein the SH2 domain contains an arginine at a position that corresponds to amino acid 601 of SEQ ID NO:2 and amino acid 602 of SEQ ID NO:4.

86. The recombinant DNA molecule of Claim 81 wherein the RRF forms a dimer upon said activation by tyrosine phosphorylation.

87. The recombinant DNA molecule of Claim 81 wherein the activation of the RRF is unaffected by the presence or concentration of second messengers.

88. The recombinant DNA molecule of Claim 81 wherein the RRF can act as a DNA binding protein upon said activation by tyrosine phosphorylation.

89. The recombinant DNA molecule of Claim 81 wherein the RRF interacts with an interferon-γ-bound receptor kinase complex.

90. The recombinant DNA molecule of Claim 88 wherein the RRF can stimulate ISRE-dependent or gamma activated site (GAS)-dependent transcription.

91. An isolated nucleic acid encoding a receptor recognition factor (RRF) protein having the following characteristics:

(a) the RRF is cytoplasmic in origin;

(b) the RRF is activated by tyrosine phosphorylation; and

(c) upon activation said RRF is translocated to the nucleus of a target cell;

wherein the RRF contains one or more of the boxed regions in Figure 8B.

92. The isolated nucleic acid of Claim 91, wherein the RRF further contains a tyrosyl residue at a position that corresponds to the conserved position identified in SEQ ID NO:2 and SEQ ID NO:4, said position selected from the group consisting of:

amino acid 22 of SEQ ID NO:2 and amino acid 22 of SEQ ID NO:4;
amino acid 34 of SEQ ID NO:2 and amino acid 33 of SEQ ID NO:4;
amino acid 288 of SEQ ID NO:2 and amino acid 289 of SEQ ID NO:4;
amino acid 631 of SEQ ID NO:2 and amino acid 634 of SEQ ID NO:4;
amino acid 648 of SEQ ID NO:2 and amino acid 651 of SEQ ID NO:4;
amino acid 665 of SEQ ID NO:2 and amino acid 668 of SEQ ID NO:4;
amino acid 677 of SEQ ID NO:2 and amino acid 680 of SEQ ID NO:4;
amino acid 678 of SEQ ID NO:2 and amino acid 681 of SEQ ID NO:4; and
amino acid 690 of SEQ ID NO:2 and amino acid 701 of SEQ ID NO:4.

93. The recombinant DNA molecule of Claim 81 that is operatively linked to an expression control sequence.

94. An expression vector containing the recombinant DNA molecule of Claim 93.

95. A method of expressing a recombinant receptor recognition factor in a cell containing the expression vector of Claim 94 comprising culturing the cell in an appropriate cell culture medium under conditions that provide for expression of the receptor recognition factor by the cell.

96. The method of Claim 95 further comprising the step of purifying the recombinant receptor recognition factor.--

REMARKS

The Specification has been amended to incorporate references to the appropriate SEQ ID NOs:, where specific sequences are indicated. Applicants have amended the Specification to note the status of the parent applications, above and have also addressed the issues

concerning 37 CFR 1.821(c). The Specification has been amended as described above, in order to correct obvious typographical errors.

At the outset, Applicants bring to the Examiner's attention the NOTICE OF INCOMPLETE APPLICATION issued on 4/20/94 in the co-pending parent application, Serial No. 08/212,185. Applicants responded on 5/26/94 with a PETITION FOR FILING DATE UNDER 37 C.F.R. § 1.53(b) which was favorably received in the DECISION ON PETITION issued by the Special Program Examiner on August 8, 1994. Copies of these papers are included in the present filing.

Support for the newly added claims can be found throughout the present Specification as indicated below, and more specifically in the Specification as originally filed on March 19, 1992, *see* lines 1-21 on Page 37 of the present Specification. Support for Claims 69-80 as related to the properties of the claimed RRFs may be found on lines 18-29 of Page 4; on lines 1-12 of Page 8; on lines 13-24 of Page 12; and throughout the first three Examples. Further support to the claimed sequence homology between SEQ ID NOs:2 and 4 may be found on lines 8-10, and 15-22 on Page 6; on lines 3-5 of Page 20; and throughout Figure 8b. Further support for Claims 79-80 may be found on lines 15-18 of Page 6; Claims 14 and 15; lines 25-27 on Page 32; on lines 6-8 of Page 35; and throughout the first three Examples. Further support for Claims 81 and 99 may be found in the Specification on line 18 of Page 4 through line 3 of Page 5, on lines 1-12 of Page 8, on lines 13-24 of Page 12, on lines 3-10 of Page 21, and in Figure 8B, on lines 10-30 of Page 35, and throughout the Examples. Further support for Claims 82 and 92 may be found on lines 8-10 of Page 21, and in Figure 8B of the Specification. Further support for Claim 83 may be found on lines 28-30 of Page 20 of the Specification. Further support for Claims 84-86 may be found in the Specification on lines 1-8 of Page 5, on lines 22-29 of Page 7, on lines 4-18 of Page 26, line 4 of Page 83 through line 27 of Page 87, and in Figures 19-23. Further support for Claim 87 may be found on Page 5, lines 10-14, and Page 7, lines 1-2 of the Specification. Further support for Claims 88-90 may be found in the Specification on Page 7, lines 9-20, on Page 8, lines 1-12, on Page 24, lines 23-29, on Page 36, lines 7-24, and in Figure 18. Further support for Claims 93-96 may be found in the Specification on lines 2-7, and 14-26, of Page 10, on lines 5-12 of Page 11, and on lines 15-18 of Page 16. Claims 1, and 69-96 remain for consideration.

Attached hereto is a marked up version of the changes made to the Specification and claims by the current amendment. The attached page is captioned "Version with marking to show changes made."

No additional fees are believed to be necessitated by the foregoing amendments. However, should this be erroneous, authorization is hereby given to charge Deposit Account No. 11-1153 for any underpayment, or credit any overages.

Applicants respectfully request entry of the foregoing amendment into the file history of the above-identified Application being filled herewith. Early and favorable action on the pending set of Claims is earnestly solicited.

Respectfully submitted,

MICHAEL D. DAVIS
Attorney for Applicant(s)
Registration No. 39,161

KLAUBER & JACKSON
411 Hackensack Avenue
Hackensack, New Jersey 07601
(201) 487-5800
Date: June 7, 2001

"VERSION WITH MARKING TO SHOW CHANGES MADE."

IN THE SPECIFICATION:

The paragraph on Page 1, beginning on line 6 directly under the subtitle: "CROSS-REFERENCE TO RELATED APPLICATIONS " has been amended as follows:

The present Application is a Continuation of copending U.S. Ser. No. 09/488,442 filed January 20, 2000 which is a Continuation of copending U.S. Ser. No. 08/948,547, filed October 10, 1997, and issued as U.S. Pat. No. 6,124,118, which is a Continuation of U.S. Serial No. 08/820,754, filed Mar. 19, 1997 and issued as U.S. Pat. No. 5,976,835, which is a Division of U.S. Ser. No. 08/212,185, filed Mar. 11, 1994 which is a Continuation-In-Part of ~~copending~~ U.S. Serial, No. 08/126,588 and ~~copending~~ U.S. Serial, No. 08/126,595, both filed Sep. 24, ~~1994~~ 1993, both now abandoned, which are both Continuations-In-Part of ~~copending~~ U.S. Serial, No. 07/980,498, filed Nov. 23, 1992, now abandoned, which is a Continuation-In-Part of U.S. Serial, No. 07/854,296, filed Mar. 19, 1992, now abandoned, the disclosures of which are hereby incorporated by reference in their entireties. Applicants claim the benefits of these Applications under 35 U.S.C. § 120.

The last paragraph on Page 5 has been amended as follows:

The recognition factor is now known to comprise several proteinaceous substituents, in the instance of IFNα and IFNγ. Particularly, three proteins derived from the factor ISGF-3 have been successfully sequenced and their sequences are set forth in FIGURE 1 (SEQ ID NOS:1, 2), FIGURE 2 (SEQ ID NOS:3, 4) and FIGURE 3 (SEQ. ID NOS.5, 6) herein. Additionally, a murine gene encoding the 91 kD protein (*i.e.*, the murine homologue of the human protein having an amino acid sequence of SEQ ID NO:4) has been identified and sequenced. The nucleotide sequence (SEQ ID NO:7) and deduced amino acid sequence (SEQ ID NO:8) are shown in FIGURE 13A-13C.

The second full paragraph of Page 7, beginning on line 9 has been amended as follows:

In a specific example, the receptor recognition factor represented by SEQ ID NO:4 possesses the added capability of acting as a ~~translation protein~~ transcription factor and, in particular, as a DNA binding protein in response to interferon-γ stimulation. This discovery presages an expanded role for the proteins in question, and other proteins and like factors that have heretofore been characterized as receptor recognition factors. It is therefore apparent that a single factor may indeed provide the nexus between the liganded receptor at the cell surface and direct participation in DNA transcriptional activity in the nucleus. This pleiotypic factor has the following characteristics:

a) It interacts with an interferon-γ-bound receptor kinase complex;

b) It is a tyrosine kinase substrate; and

c) When phosphorylated, it serves as a DNA binding protein.

The bridging paragraph between Pages 8 and 9, beginning on line 29 of Page 8 has been amended as follows:

The present invention also relates to a recombinant DNA molecule or cloned gene, or a degenerate variant thereof, which encodes a receptor recognition factor, or a fragment thereof, that possesses a molecular weight of about 113 kD and an amino acid sequence set forth in FIGURE 1 (SEQ ID NO:2); preferably a nucleic acid molecule, in particular a recombinant DNA molecule or cloned gene, encoding the 113 kD receptor recognition factor has a nucleotide sequence or is complementary to a DNA sequence shown in FIGURE 1 (SEQ ID NO:1). In another embodiment, the receptor recognition factor has a molecular weight of about 91 kD and the amino acid sequence set forth in FIGURE 2 (SEQ ID NO:4) or FIGURE 13 (SEQ ID NO:8); preferably a nucleic acid molecule, in particular a recombinant DNA molecule or cloned gene, encoding the 91 kD receptor recognition factor has a nucleotide sequence or is complementary to a DNA ~~seqnece~~ sequence shown in FIGURE 2 (SEQ ID NO:3) or FIGURE 13 (SEQ ID NO:8). In yet a further embodiment, the receptor recognition factor has a molecular weight of about 84 kD and the amino acid sequence set forth in FIGURE 3 (SEQ ID NO:6); preferably a nucleic acid molecule, in particular a recombinant DNA molecule or cloned gene, encoding the 84 kD receptor recognition factor

has a nucleotide sequence or is complementary to a DNA ~~seqnece~~ sequence shown in FIGURE 3 (SEQ ID NO:5). In yet another embodiment, the receptor recognition factor has an amino acid sequence set forth in FIGURE 14 (SEQ ID NO:10); preferably a nucleic acid molecule, in particular a recombinant DNA molecule or cloned gene, encoding such receptor recognition factor has a nucleotide sequence or is complementary to a DNA ~~seqnece~~ sequence shown in FIGURE 14 (SEQ ID NO:9). In still another embodiment, the receptor recognition factor has an amino acid sequence set forth in FIGURE 15 (SEQ ID NO:12); preferably a nucleic acid molecule, in particular a recombinant DNA molecule or cloned gene, encoding such receptor recognition factor has a nucleotide sequence or is complementary to a DNA ~~seqnece~~ sequence shown in FIGURE 15 (SEQ ID NO:11).

The fifth paragraph of Page 17, beginning on line 23 has been amended as follows:

- - FIGURE 1A-1E depicts the full receptor recognition factor nucleic acid sequence and the deduced amino acid sequence derived for the ISGF-3α gene defining the 113 kD protein. The nucleotides are numbered from 1 to 2553 (SEQ ID NO:1), and the amino acids are numbered from 1 to 851 (SEQ ID NO:2).

The sixth paragraph of Page 17, beginning on line 28 has been amended as follows:

- - FIGURE 2A-2D depicts the full receptor recognition factor nucleic acid sequence and the deduced amino acid sequence derived for the ISGF-3α gene defining the 91 kD protein. The nucleotides are numbered from 1 to 3943 (SEQ ID NO:3), and the amino acids are numbered from 1 to 750 (SEQ ID NO:4).

The first paragraph of Page 18, beginning on line 1 has been amended as follows:

- - FIGURE 3A-3C depicts the full receptor recognition factor nucleic acid sequence and the deduced amino acid sequence derived for the ISGF-3α gene defining the 84 kD protein. The nucleotides are numbered from 1 to 2166 (SEQ ID NO:5), and the amino acids are numbered from 1 to 712 (SEQ ID NO:6). - -

The third paragraph of Page 18, beginning on line 19 has been amended as follows:

- - FIGURE 5a-5b generally presents the results of Northern Blot analysis for the 91/84 kD peptides. Figure 5a presents restriction maps for cDNA clones E4 (top map) and E3 (bottom map) showing DNA fragments that were radiolabeled as probes (probes A-D). Figure 5b comprises Northern blots of cytoplasmic HeLa RNA hybridized with the indicated probes. The 4.4 and 3.1 KB species as well as the 28S and 18S rRNA bands are indicated.

The first full paragraph of Page 19, beginning on line 4 has been amended as follows:

- - FIGURE 7a-7e presents the results of Western blot and antibody shift analyses.

a) Highly purified ISGF-3, fractionated on a 7.0% SDS polyacrylamide gel, was probed with antibodies a42 (amino acids 597-703); a55 (amino acids 2-59); and a57 (amino acids 705-739) in a Western blot analysis. The silver stained part of the gel (lanes a, b, and c) illustrates the location of the ISGF-3 component proteins and the purity of the material used in Western blot: Lane a) Silver stain of protein sample used in all the Western blot experiments (immune and preimmune). Lane b) Material of equal purity to that shown in Fig. 4, for clearer identification of the ISGF-3 proteins. Lane c) Size protein markers indicated.

b) Antibody interference of the ISGF-3 shift complex; Lane a) The complete ISGF-3 and the free ISGF-3γ component shift with partially purified ISGF-3 are marked; Lane b) Competition with a 100 fold excess of cold ISRE oligonucleotide. Lane c) Shift complex after the addition of 1 ml of preimmune serum to a 12.5 µl shift reaction. Lanes d and e) - Shift complex after the addition of 1 µl of a 1:10 dilution or 1 ml of undiluted a42 antiserum to a 12.5 µl shift reaction. - -

The first full paragraph of Page 23, beginning on line 4 has been amended as follows:

- -FIGURE 13 depicts (A) the deduced amino acid sequence (SEQ ID NO:8) of and (B- C ~~D~~) the DNA sequence (SEQ ID NO:7) encoding the murine 91 kD intracellular receptor recognition factor. - -

The second full paragraph of Page 23, beginning on line 8 has been amended as follows:

- - FIGURE 14 depicts (A) the deduced amino acid sequence (SEQ ID NO:10) of and (B-D ~~C~~) the DNA sequence (SEQ ID NO:9) encoding the 13sf1 intracellular receptor recognition factor.

The third full paragraph of Page 23, beginning on line 12 has been amended as follows:

- - FIGURE 15 depicts (A) the deduced amino acid sequence (SEQ ID NO:12) of and (B-E~~C~~) the DNA sequence (SEQ ID NO:11) encoding the 19sf6 intracellular receptor recognition factor. - -

The bridging paragraph between Pages 37 and 38, beginning on line 23 of Page 37 has been amended as follows:

- - As stated above, the present invention also relates to a recombinant DNA molecule or cloned gene, or a degenerate variant thereof, which encodes a receptor recognition factor, or a fragment thereof, that possesses a molecular weight of about 113 kD and an amino acid sequence set forth in FIGURE 1 (SEQ ID NO:2); preferably a nucleic acid molecule, in particular a recombinant DNA molecule or cloned gene, encoding the 113 kD receptor recognition factor has a nucleotide sequence or is complementary to a DNA sequence shown in FIGURE 1 (SEQ ID NO:1). In another embodiment, the receptor recognition factor has a molecular weight of about 91 kD and the amino acid sequence set forth in FIGURE 2 (SEQ ID NO:4) or FIGURE 13 (SEQ ID NO:8); preferably a nucleic acid molecule, in particular a recombinant DNA molecule or cloned gene, encoding the 91 kD receptor recognition factor has a nucleotide sequence or is complementary to a DNA ~~seqnece~~ sequence shown in FIGURE 2 (SEQ ID NO:3) or FIGURE 13 (SEQ ID NO:8). In yet a further embodiment, the receptor recognition factor has a molecular weight of about 84 kD and the amino acid sequence set forth in FIGURE 3 (SEQ ID NO:6); preferably a nucleic acid molecule, in particular a recombinant DNA molecule or cloned gene, encoding the 84 kD receptor recognition factor has a nucleotide sequence or is complementary to a DNA ~~seqnece~~ sequence shown in FIGURE 3 (SEQ ID NO:5). In yet another embodiment, the receptor recognition factor has an amino acid sequence set forth in FIGURE 14 (SEQ ID NO:10); preferably a

nucleic acid molecule, in particular a recombinant DNA molecule or cloned gene, encoding such receptor recognition factor has a nucleotide sequence or is complementary to a DNA ~~seqnece~~ sequence shown in FIGURE 14 (SEQ ID NO:9). In still another embodiment, the receptor recognition factor has an amino acid sequence set forth in FIGURE 15 (SEQ ID NO:12); preferably a nucleic acid molecule, in particular a recombinant DNA molecule or cloned gene, encoding such receptor recognition factor has a nucleotide sequence or is complementary to a DNA ~~seqnece~~ sequence shown in FIGURE 15 (SEQ ID NO:11). - -

The bridging paragraph between Pages 69 and 70, beginning on line 30 of Page 69 has been amended as follows:

- - A fragment of the gene encoding the human 91 kD protein was used to screen a murine thymus and spleen cDNA library for homologous proteins. The screening assay yielded a highly homologous gene encoding a murine polypeptide that is greater than 95% homologous to the human 91 kD protein. The nucleic acid and deduced amino acid sequence of the murine 91 kD protein are shown in Figure ~~12A-12C~~ 13A-13C, and SEQ ID NO:7 (nucleotide sequence) and SEQ ID NO:8 (amino acid sequence). - -

Page 76, line 11, following the second paragraph has been amended as follows:

EXAMPLE 6: DIMERIZATION OF PHOSPHORYLATED STAT91

The title of the application has also been amended to read:

NUCLEIC ACIDS ENCODING RECEPTOR RECOGNITION FACTORS, ~~PROTEIN SEQUENCES~~ AND METHODS OF USE THEREOF

The Applicants have also requested that the Specification be amended to include the Sequence Listing submitted herewith and enclose a copy of the Sequence Listing for the Examiner's convenience.

<u>IN THE CLAIMS</u>:

Claims 2-68 have been canceled without prejudice.

Claims 69-96 have been added.